REMARKS

Entry of the foregoing Amendment is requested. As of entry of the Amendment, claims 1-36 will be pending.

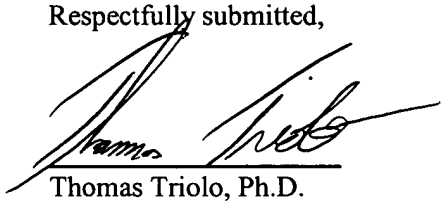
Each amendment has written support in the specification, as filed; accordingly, no new matter has been added to the Application. The Amendment corrects the SEQ ID NO. which corresponds to each gene product in Tables 1a, 1b and 2-7 and at several other places in the specification. Written support for these changes appears, for example, in the Sequence Listing. The other changes to the specification are only formal changes which, as stated above, do not add any new matter.

Claims 1, 2, 4-12, 19-21, 23, 24 and 26-28 have been amended and new claims 35 and 36 have been added. Claims 2 and 5 have been amended to identify the recited nucleotide and amino acid sequences by SEQ ID NO. Claim 19 have been amended to correct the recited SEQ ID NOs. Written support for amended claims 2, 5 and 19 appears, for example, in Tables 1a and 1b and in the Sequence Listing. The other claim amendments make only formal changes; written support for these amended claims appears, for example, in the claims as filed. Written support for new claim 35 appears, for example, at claim 4, as filed. Written support for new claim 36 appears, for example, at page 5, lines 24-25; at figure 7B and at the Sequence Listing (SEQ ID NO: 177).

CONCLUSION

Early and favorable action is earnestly solicited.

Respectfully submitted,



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File No. ID0983 K US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Hosted *et al.*

Serial No.: 09/758,759 Group Art Unit: 1635

Filed: January 11, 2001 Examiner: To be assigned

For: EVERNINOMICIN BIOSYNTHETIC GENES

MARKED-UP AMENDMENT UNDER 37 C.F.R §1.121

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

The present Marked-Up Amendment shows all changes made to the specification in the enclosed Preliminary Amendment.

IN THE CLAIMS: ***Please amend claims 1, 2, 4-12, 19-21, 23, 24 and 26-28 as follows:***

1 (Amended). An isolated nucleic acid [comprising] encoding an everninomicin biosynthetic pathway gene product from a *Micromonospora carbonacea*.

2 (Amended). [The] An isolated nucleic acid encoding a polypeptide comprising an amino acid sequence[of claim 1, which encodes a gene product] selected from [everninomicin biosynthetic enzymes and proteins listed in Tables 1a and 1b] SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202 and 204.

4 (Amended). [The] An isolated nucleic acid comprising [of claim 3, which comprises] a coding sequence from the nucleotide sequence [as depicted in] of SEQ ID NO:1[, or the complement thereof].

5 (Amended). The isolated nucleic acid of claim 4[, wherein the] comprising a nucleotide sequence [is] selected from [the group consisting of CDS sequences listed in Tables 1a and 1b] SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201 and 203.

6 (Amended). The isolated nucleic acid of claim [1] 2, wherein the gene product is involved in orsellinic acid biosynthesis.

7 (Amended). The isolated nucleic acid of claim [1] 2, wherein the gene product is a sugar biosynthetic gene product.

8 (Amended). The isolated nucleic acid of claim [1] 2, wherein the gene product is a glycosyltransferase.

9 (Amended). The isolated nucleic acid of claim [1] 2, wherein the gene product is a tailoring enzyme.

10 (Amended). The isolated nucleic acid of claim [1] 2, wherein the gene product is a regulatory gene product.

11 (Amended). The isolated nucleic acid of claim [1] 2, wherein the gene product is involved in a resistance mechanism.

12 (Amended). An expression vector comprising a nucleic acid of claim [1] 2 operably associated with an expression control sequence.

19 (Amended). [The] An isolated polypeptide [of claim 18, which comprises] comprising an amino acid sequence selected from [SEQ ID NOS:2-88 and 96-106] SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151,

153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202 and 204.

20 (Amended). A modified *Micromonospora carbonacea*, wherein an everninomicin biosynthetic pathway gene is knocked-out or over-expressed.

21 (Amended). A modified *Micromonospora carbonacea*, wherein an everninomicin biosynthetic pathway gene according to claim 2 is knocked-out or over-expressed.

23 (Amended). A vector comprising a nucleic acid encoding a *Micromonospora carbonacea* everninomicin biosynthetic pathway resistance gene product according to claim 19.

24 (Amended). A method [of] for selecting for a transfected or transformed host cell, comprising selecting a host cell containing the vector of claim 23 and cultured in the presence of an amount of everninomicin that is toxic to the host cell which does not contain the vector.

26 (Amended). [The] An isolated nucleic acid [of claim 25, wherein the site-specific integrase has an amino acid sequence as depicted in] encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:[89] 177.

27 (Amended). [The] An isolated nucleic acid [of claim 26, wherein the site-specific integrase has a nucleotide sequence as depicted in] comprising the nucleotide sequence of SEQ ID NO:[90] 176.

28 (Amended). A vector for integration in an actinomycete host cell comprising the nucleic acid of claim [25] 26.

IN THE SPECIFICATION:

Please replace pages 7-13 in the present specification with the following pages:

chromosome. This locus is depicted in Figures 2A and 3. Since everninomicin is only known to be produced in *M. carbonacea*, for the sake of particularity the EV biosynthetic pathway is associated with this microorganism. However, it should be understood that this term encompasses EV biosynthetic enzymes (and genes encoding such enzymes) isolated from any *M. carbonacea*, and furthermore that these genes may have novel homologues in related actinomycete bacteria that fall within the scope of the claims here. In specific embodiments, these genes are depicted in Figure 11 (SEQ ID NO:1; open reading frames and polypeptides designated as SEQ ID NOS:[2-88] 2-175) and Figure 12 (SEQ ID NO:[95] 182; open reading frames and polypeptides designated as SEQ ID NOS:[96-106] 183-204). It is noted that the sequences of Figures 11 and 12 are linked (contiguous) or connected such that they are part of the same cluster, *i.e.*, the sequence in Figure 12 precedes that of Figure 11. Moreover, the present inventors have identified specific categories into which many of the genes from the EV biosynthetic pathway fall, including but by no means limited to, orsellinic acid biosynthetic enzymes, sugar biosynthetic enzymes, glycosyltransferases, tailoring enzymes, regulatory enzymes (serine-threonine kinases), and resistance mechanism enzymes (rRNA methylases and transporter enzymes). These categories are discussed in greater detail, *infra*. The gene products are listed in Tables 1a and 1b.

Table 1a. Gene Products and Putative Enzymatic Functions Involved in Everninomicin Production

Gene Product	CDS[1]	RBS[2]	SEQ ID NO.[4]	Enzymatic Function (Protein ACC No; BLAST Score)	Class
evdA length 416aa [417aa]	(132...1382)*	(1389...1394)*	2, 3	similarity to hydroxylase (CAA11782; 6.5e-137)	sugar biosynthetic
evdB length 373aa [474aa]	(1490...2611)*	(2618...2622)*	[3]4, 5	hexose aminotransferase, dnrJ homolog (daunorubicin) (P25048; 2.8e-65)	sugar NH2 addition
evdC length 412aa [413aa]	(2622...3860)*	(3867...3870)*	[4]6, 7	similar to flavoprotein, oxidase (S39965; 4.4e-92)	sugar biosynthetic
evdD length 389aa [390aa]	(4143...5312)	(4134...4138)	[5]8, 9	dNTP-hexose glycosyltransferase (AAC01731; 4.6e-49)	Glycosyl transfer
evdE length 308aa	(5309...6235)		[6]10, 11	hexose dehydratase (CAA18814; 8.0e-58)	sugar biosynthetic
evdF length 347aa	(6232...7275)	(6226...6229)	[7]12, 13	dNTP-hexose glycosyltransferase (CAB07092; 3.4e-18)	Glycosyl transfer
evdG length 351aa	(7272...8327)		[8]14, 15	unknown	unknown

evdH length 340aa	(8342...9364)	(8333...8336)	[9]16, 17	dNTP-hexose glycosyltransferase (CAA19930; 0.8)	Glycosyl transfer
evdI length 253aa	(9463...10,224)*	(10,232...10,235)*	[10]18, 19	hydrolase (AAB81835; 6.8e-10)	sugar biosynthetic
evdJ length 250aa	(10,424...11,176)		[11]20, 21	unknown	unknown
evdK length 415aa	(11,208...12,455)		[12]22, 23	hexose dehydratase or empimerase (CAB08849; 3.3e-26)	sugar biosynthetic
evdL length 304aa	(12,108...13,022)*	(13,027...13,030)*	[13]24, 25	dNTP-hexose glycosyltransferase (S37028; 0.010)	Glycosyl transfer
evrA length 317aa	(14,410...15,363)*	(15,369...15,373)*	[14]26, 27	hexose epimerase (CAA12010.1; 1.3e-40)	sugar biosynthetic
evrB length 344aa	(15,380...16,414)*		[15]28, 29	hexose oxidoreductase (ACC01734; 1.3e-65)	sugar biosynthetic
evrC length 484aa	(16,419...17,873)*		[16]30, 31	hexose dehydratase (CAA12009; 2.2e-107)	sugar biosynthetic
evrD length 354aa	(17,870...18,934)*		[17]32, 33	GDP-mannose 4,6-dehydratase (BAA16585; 1.0e-88)	sugar biosynthetic
evrE length 510aa	(19,374...20,906)		[18]34, 35	multidrug efflux transporter (CAB15277; 1.4e-59)	resistance mechanism
evrF length 492aa	(21,064...22,542)	(21,056...22,542)	[19]36, 37	similar to non-heme oxygenate/halogenase (CAA11780; 4.3e-58)	orsellinic acid chlorine addition
evrG length 474aa	(22,748...24,172)	(22,736...22,740)	[20]38, 39	oxidase (Q12737; 5.5e-67)	tailoring
evrH length 348aa	(24,177...25,223)*	(25,230...25,233)*	[21]40, 41	unknown (AAB89073; 3.2e-6)	unknown
evrI length 358aa	(25,550...26,626)		[22]42, 43	acyl starter unit fidelity (daunorubicin homology) (AAA65208; 5.7e-56)	PKS acyl Carbon choice
evrJ length 1264aa	(26,685...30,479)	(26,672...26,676)	[23]44, 45	orsellinic acid synthase 6-methylsalicilic acid synthetase (CAA72713; 0.0e)	polyketide synthetase
evrK length 439aa	(30,557...31,876)*	(31,885...31,888)*	[24]46, 47	Na/H antiporter (BAA16991; 2.1e-14)	unknown
evrL length 313aa	(31,941...32,882)*		[25]48, 49	similar to gene essential to heme biosynthesis (BAA12681; 0.0012)	unknown
evrM length 412aa	(33,167...34,405)*	(34,414...34,418)*	[26]50, 51	similar to p450 hydroxylase (S18530; 3.8e-70)	tailoring

evrN length 253aa	(34,449...35,210)*	(35,219...35,221)*	[27]52, 53	methyl transferase (CAB10751; 0.00061)	tailoring
evrO length 314aa	(35,294...36,238)*		[28]54, 55	unknown (BAA20094; 0.56)	unknown
evrP length 242aa	(36,235...36,963)*		[29]56, 57	unknown (CAB05421; 0.00020)	unknown
evrQ length 342aa	(36,998...38,026)*		[30]58, 59	similar to oxidoreductase and heat stress protein (P80874; 7.8e-31)	tailoring
evrR length 164aa	(38,072...38,566)*		[31]60, 61	low similarity to hexaheme nitrite reductase regulator (P30866; 0.0034)	regulatory (methyl transferase)
evrS length 423aa	(38,892...40,163)*		[32]62, 63	dNTP-hexose glycosyltransferase (AAD15267; 1.9e-36)	Glycosyl transfer
evrT length 224aa	(40,216...40,890)*	(40,899...40,902)*	[33]64, 65	similar to L-proline hydroxylase (BAA 20094; 5.5e-7)	tailoring
evrU length 229aa	(40,887...41,576)*		[34]66, 67	methyltransferase (CAB02029; 5.6e-6)	tailoring
evrV length 342aa	(41,679...42,707)*	(42,714...42,717)*	[35]68, 69	dTDP-glucose epimerase (AAB84886; 3.5e-36)	L-dTDP-glucose biosynthetic
evrW length 329aa [333aa]	(42,810...43,799)*	(43,807...43,811)*	[36]70, 71	dTDP-glucose dehydratase (CAA72715; 5.1e-136)	D-dTDP-glucose biosynthetic (GDH)
evrX length 355aa	(43,799...44,866)*		[37]72, 73	dTDP-glucose synthetase (A26984; 1.2e-118)	D-dTDP-glucose biosynthetic
evrY length 248aa	(45,014...45,760)*	(45,767...45,770)*	[38]74, 75	dehalogenase (P24069; 5.8e-8)	drug resistance
evrZ length 250aa	(45,962...46,714)*	(45,952...45,956)*	[39]76, 77	similar to muramidase/lysozyme (P25310; 1.2e-77)	drug resistance
evsA length 692aa	(47,156...49,234)*		[40]78, 79	serine threonine kinase (BAA32455; 2.0e-76)	regulatory
evsB length 362aa [363aa]	(51,627...52,715)	(51,620...51,622)	[41]80, 81	similar to proteases	unknown
evsC length 222aa	(52,889...53,557)		[42]82, 83	similar to MAF involved in septum formation (BAA18425; 1.3e-21)	unknown
evbA length 217aa	(53,554...54,207)		[43]84, 85	O-methyl transferase (AAC44130; 8.6e-38)	tailoring; possible resistance
evbB length 251aa	(54,362...55,117)*	(55,125...55,128)*	[44]86, 87	membrane pump, homolog mithramicin resistance (AAC443581; 2.9e-24)	resistance mechanism

evbC length 319aa	(55,135...56,094)*	(56,100...56,103)*	[45]88, 89	membrane pump, homolog mithramicin resistance (AAC44357; 1.0e-69)	resistance mechanism
evbC2 length 198aa [209aa]	(56,184...56,813)*		[46]90, 91	ankrylin like (AAC44356; 0.0041)	resistance
evbD length 582aa	(56,961...58,709)	(56,947...56,951)	[47]92, 93	acyl-CoA carboxylase (CAB07068; 7.3e-201)	malonyl-CoA biosynthesis
evbE length 479aa	(58,873...60,312)		[48]94, 95	IMP dehydrogenase (CAA15452; 4.1e-165)	tailoring
evbF length 185aa	(60,472...61,029)*	(61,038...61,040)*	[49]96, 97	hypothetical protein Rv0653c, mycobacterium (CAB07128; 3.8e-06)	regulator
evbF1 length 90aa	(61,288...61,560)		[50]98, 99	unknown	unknown
evbF2 length 152aa	(61,610...62,069)	(61,597...61,599)	[51]100, 101	ORFI Streptomyces peucetius (CAA06602; 0.024)	regulatory/ resistance
evbG length 557aa	(62,122...63,795)		[52]102, 103	ABC transporter (Q11046; 2.7e-170)	drug resistance
evbH length 645aa	(63,891...65,828)	(63,884...63,887)	[53]104, 105	ABC transporter (Q11047; 5.6e-166)	drug resistance
evbI length 467aa	(66,469...67,872)*	(67,883...67,886)*	[54]106, 107	lipoamide dehydrogenase (CAA17075; 1.6e-140)	tailoring
evbJ length 151aa	(67,979...68,434)		[55]108, 109	hypothetical protein Rv3304 [Mycobacterium tuberculosis] (CAA17076; 7.6e-40)	unknown
evbK length 321aa	(68,529...69,494)		[56]110, 111	protease synthase and sporulation regulator; homology to resistance proteins streptomyces (029729; 7.3-7)	regulatory
evbL length 249aa	(69,610...70,359)*		[57]112, 113	acetyltransferase/ phosphotransferase	tailoring
evbM length 306aa	(70,365...71,285)*		[58]114, 115	hypothetical protein Rv 1584c [Mycobacterium tuberculosis] (CAB09085; 0.32)	unknown
evbN length 209aa	(71,289...71,918)*	(71,926...71,929)*	[59]116, 117	hypothetical protein SC3A7.08 [S. coelicolor] (CAA20071; 4.0e-40)	unknown
evbO length 230aa [231aa]	(72,284...72,979)		[60]118, 119	putative lipoprotein [S. coelicolor] (CAA19252; 2.6e-20)	unknown
evbP length 420aa	(72,933...74,195)*		[61]120, 121	peptidase (CAA17077; 6.5e-88)	unknown

evbQ length 527aa	(74,707...76,290)*		[62]122, 123	methylmalonyl-Coa mutate (BAA30410; 1.8e-149)	acyl precursor biosynthesis
evbR length 696aa	(76,622...78,712)		[63]124, 125	protein serine/threonine kinase note eukaryotic type (BAA32455; 1.1e-71)	regulatory
evbS length 576aa	(78,791...80,521)		[64]126, 127	phosphomannomutase (CAA17080; 5.4e-91)	sugar biosynthesis
evbT length 286aa	(82,073...82,933)		[65]128, 129	hypothetical protein SC5C7.22c (CAA20634; 5.7e-28)	10-28
evbU length 202aa	(83,280...83,888)*		[66]130, 131	glucose-6-phosphate 1-dehydrogenase low BLAST homology (S61167; 0.00039)	unknown
evbV length 193aa	(84,080...84,661)*		[67]132, 133	uracil phosphoribosyl transferase (CAA17081; 5.6e-60)	unknown
evbW length 338aa	(84,890...85,906)*		[68]134, 135	deoxyribose-phosphate aldolase (AAA79343; 1.3e-54)	unknown
evbX length 477aa	(85,909...87,342)		[69]136, 137	aldehyde dehydrogenase (AAB84440; 4.2e-103)	tailoring
evbY length 245aa [735aa]	(87,422...88,159)	(87,407...87,411)	[70]138, 139	aldehyde dehydrogenase (CAA71003; 3.4e-16)	tailoring
evbZ length 137aa	(88,292...88,705)	(88,280...88,282)	[71]140, 141	hypothetical protein (CAB06141; 1.3e-16)	unknown
evcA length 301aa	(88,716...89,621)		[72]142, 143	hypothetical protein, putative integral membrane protein [Streptomyces coelicolor] (CAB06143; 4.5e-28)	unknown
evcB length 416aa	(89,817...91,067)		[73]144, 145	cytochrome D oxidase subunit I (P94364; 3.0e-65)	tailoring
evcC length 335aa	(91,078...92,085)	(91,068...91,072)	[74]146, 147	cytochrome D oxidase subunit II (CAA71118; 1.9e-15)	tailoring
evcD length 561aa	(92,148...93,833)		[75]148, 149	ABC transporter (CAA22219; 2.6e-107)	resistance
evcE length 613aa	(93,830...95,671)		[76]150, 151	ABC transporter (AAC44070; 3.4e-32)	resistance
evcF length 229aa	(95,729...96,418)		[77]152, 153	unknown	unknown
evcG length 111aa	(96,440...96,775)*		[78]154, 155	unknown (AAB84787; 1.9e-8)	unknown
evcH length 303aa	(96,894...97,805)		[79]156, 157	unknown (CAA17083; 9.2e-5)	unknown

evcI search length 691aa	(98,287...100,362)		[80]158, 159	unknown (CAA19992; 6.0e-6)	unknown
evcJ length 197aa	(100,733...101,326)*		[81]160, 161	putative ATP/GTP binding protein (CAA19989; 7.9e-59)	unknown
evcJ2 length 134aa	(101,328...101,732)*		[82]162, 163	unknown (CAA19986; 8.6e-23)	unknown
evcK length 117aa	(101,803...102,156)*		[83]164, 165	unknown (CAA19991; 1.7e-36)	unknown
evcL search length 1145aa	(102,204...105,641)*		[84]166, 167	unknown (CAA19992; 4.6e-99)	unknown
evcM length 201aa [88aa]	(105,907...105,641)		[85]168, 169	putitive uridine kinase (CAA19591; 1.0e-9)	unknown
evcN length 358aa	(106,513...107,589)		[86]170, 171	unknown (CAA17085; 7.5e-120)	unknown
evrMR length 320aa	(107,653...108,615)	(107,637...107,641)	[87]172, 173	homology to 23S rRNA methylase for mycinamicin resistance (myrA) (BAA03674; 1.4e-79)	resistance
evrMR2 [Length] length 193aa	(108,635...109,216)		[88]174, 175	homology to gene linked to myrA	resistance

Table 1b. Gene Products and Putative Enzymatic Functions Involved in Everninomicin Production

ORF1 length 291aa	(189-1064)*	(1069-1073)	[96]183, 184	Transcriptional regulator Biotinylation H70979; 8e-31	unknown
ORF2 length 527aa [528]	(1184-2767)*		[97]185, 186	Propionyl-CoA carboxylase T42208; 0.0e	unknown
ORF3 length 296aa [297]	(2863-3753)*		[98]187, 188	unknown	unknown
ORF4 length 166aa [167]	(3776-4276)*	(4280-4284)	[99]189, 190	ECF sigma factor T36644; 8e-26	regulation
ORF5 length 280aa [281]	(4526-5368)*		[100]191, 192	Membrane protein CAB94598.1; 5e-50	unknown
ORF6 length 251aa [252]	(5392-6147)*	(6152-6156)	[101]193, 194	rRNA methyltransferase AAG32067.1; 4e-49	resistance
ORF7 length 362aa [363]	(6194-7282)*		[102]195, 196	O-methyl transferase PP42712; 4e-59	modification
ORF8 length 284aa	(7280-8133)	(8141-8145)	[103]197, 198	unknown	unknown

ORF9 length 354aa [355]	(8254-9318)	(9324-9328)	[104]199, 200	oxidoreductase AAG05128.1; 3e-51	modification
ORF10 length 309aa	(9575-10,504)	(9568-9571)	[105]201, 202	unknown	unknown
ORF11 Length 333aa [334]	(10,584-11,585)		[106]203, 204	deoxyhexose ketoreductase T17473; 1e-49	sugar modification

Legend for Tables 1a and 1b

* CDS, RBS complement on full length biosynthetic locus sequence

[1] CDS is then putative coding sequence.

[2] RBS is the putative ribosome binding site.

[3] GenBank protein database (http://www.ncbi.nih.gov/Entrez/protein.html)

[4] The first number corresponds to the nucleotide sequence and the second number corresponds to the amino acid sequence.

Although the term "enzymes" is used to refer to the EV biosynthetic pathway gene products, such gene products may be proteins with non-enzymatic functions. Such proteins are also contemplated as falling within the scope of the present invention.

An "EV biosynthetic pathway bottleneck gene" is a gene encoding a product whose level limits the rate of synthesis of everninomicin. Examples of such gene products include, though are not limited to, evrJ (involved in orsellinic acid biosynthesis); evrV, evrW, and evrX (involved in dTDP-glucose synthesis); evbD (involved in malonyl-CoA-synthesis, which is required for orsellinic acid synthesis); and oxidases responsible for oxidation of the amino group on the terminal sugar to produce everninomicin that contains a nitrososugar group. Other likely bottleneck genes include those encoding glycosyltransferases (evdD, evdF, evdH, evdL, and evrS) and tailoring enzymes, particularly sugar modification enzymes.

A modified *Micromonospora carbonacea* refers to a microorganisms that has been genetically engineered to over-express or suppress expression of an EV biosynthetic pathway gene product (enzyme). Such genetic engineering and manipulation is described in detail, *infra*. Preferably, to increase the level of production of everninomicin, the modified microorganism overexpresses one or more bottleneck genes. To produce an everninomicin analog or homolog, various tailoring enzyme genes (*e.g.*, evdB, a hexose aminotransferase that produces an amino sugar; evrF, a nonheme halogenase that chlorinates the orsinillic acid; or an oxidase gene that produces a nitrososugar by oxidation of an aminosugar) may be knocked out. Other knock-outs may be made of putative key genes, resulting in all likelihood in blockage of everninomicin biosynthesis. These include the orsellinic acid synthase (evrJ), dTDP-glucose synthases (evrV, evrW, and evrX), and glycosyltransferases (evdD, evdF, evdH, evdL, and evrS). A knockout of the glycosyltransferase that adds the terminal glycosyl group is expected to produce an everninomicin analog lacking the terminal glycosyl group.- -

Please replace the 5th full paragraph of page 4 (lines 20-22) with the following paragraph:

- -In addition, isolated polypeptides corresponding to an everninomicin biosynthetic pathway gene product are provided. Specific open reading frames and amino acid sequences of the polypeptides are set forth in Figure 11 (SEQ ID NOS: 2-175 [2-88]) and Figure 12 (SEQ ID NOS: 183-204 [96-106]).- -

Please replace the 7th full paragraph of page 5 (lines 24-25) with the following paragraph:

- -**Figure 7A-B**. **(A)** Map of pSPRH840 integrating vector. **(B)** Sequence of integrase gene (SEQ ID NO:[89] 176) and deduce amino acid (SEQ ID NO:[90] 177).- -

Please replace the 11th full paragraph of page 5 (lines 32-33) with the following paragraph:

- -**Figure 11A-AB**. Everninomicin biosynthetic pathway locus sequence (SEQ ID NO:1) with open reading frames and deduced amino acid sequences (SEQ ID NOS:[2-88] 2-175).- -

Please replace the 12th full paragraph of page 5 (lines 34-35) with the following paragraph:

- -**Figure 12A-G**. Everninomicin biosynthetic pathway locus sequence (SEQ ID NO:[95] 182) with open reading frames and deduced amino acid sequences (SEQ ID NOS:[96-106] 183-204).- -

Please replace the 3rd full paragraph of page 14 (lines 16-20) with the following paragraph:

- -A *Micromonospora* site-specific Att/Int functions consist of an integrase protein and AttP site, *e.g.*, as depicted in Figure 7B (SEQ ID NO:[89] 177) and in a specific embodiment encoded by a nucleic acid having a sequence as depicted in Figure 7B (SEQ ID NO:[90] 176), that permits site-specific integration of a vector into an actinomyce, and particularly a *Micromonospera*, genome.- -

Please replace Table 2 on page 32 with the following Table:

- -Table 2. Orsellinic Acid Biosynthetic Gene Products

Gene Product	CDS	SEQ ID No.	Enzymatic Function
evrF	21,064...22,542	[19] 36,37	non-heme oxygenase/halogenase addition
evrI	25,550...26,626	[22] 42,43	acyl starter unit
evrJ	26,685...30,479	[23] 44,45	orsellinic acid synthase/6-methylsalicilic acid synthase
evbD	56,961...58,709	[47] 92,93	acyl-CoA carboxylase
evbQ	74,707...76,290*	[62] 122,123	Methylmalonyl-CoA mutase

- -

Please replace Table 3 on page 34 with the following Table:

- -Table 3. Sugar Biosynthetic Gene Products

Gene Product	CDS	SEQ ID No.	Enzymatic Function
evdA	132...1382*	[2] 2, 3	hydroxylase
evdB	1490...2611*	[3] 4, 5	hexose aminotransferase
evdC	2622...3860*	[4] 6, 7	oxidase (flavoprotein)
evdE	5309...6235	[6] 10, 11	hexose dehydratase
evdI	9463...10,224*	[10] 18, 19	hydrolase
evdK	11,208...12,455	[12] 22, 23	hexose dehydratase or epimerase
evrA	14,410...15,363*	[14] 26, 27	hexose epimerase
evrB	15,380...16,414*	[15] 28, 29	hexose oxidoreductase
evrC	16,419...17,873*	[16] 30, 31	hexose dehydratase
evrD	17,870...18,934*	[17] 32, 33	GDP-mannose 4,6-dehydratase
evrV	41,679...42,707*	[35] 68, 69	dTDP-glucose epimerase
evrW	42,810...43,799*	[36] 70, 71	dTDP-glucose dehydratase
evrX	43,799...44,866	[37] 72, 73	dTDP-glucose synthetase

- -

Please replace the remainder of Table 3 on page 35 with the following Table: - -

evbS	78,791...80,521	[64] 126, 127	Phosphomannomutase
evbU	83,280...83,888	[66] 130, 131	Glucose-6-phosphate 1-dehydrogenase
ORF9	8254...9318	[104] 199, 200	Oxidoreductase
ORF11	10,584...11,585	[106] 203, 204	Deoxyhexose ketoreductase

- -

Please replace Table 4 on page 35 with the following Table:

--Table 4. Glycosyltransferases

Gene Product	CDS	SEQ ID No.	Enzymatic Function
evdD	4143...5312	[5] 8, 9	DNTP-hexose glycosyltransferase
evdF	6232...7275	[7] 12, 13	DNTP-hexose glycosyltransferase
evdH	8342...9364	[9] 16, 17	DNTP-hexose glycosyltransferase
evdL	12,108...13,022*	[13] 24, 25	DNTP-hexose glycosyltransferase
evrS	38,892...40,163*	[32] 62, 63	DNTP-hexose glycosyltransferase

Please replace Table 5 on page 36 with the following Table:

--Table 5. Tailoring Gene Products

Gene Product	CDS	SEQ ID No.	Enzymatic Function
evrG	22,748...24,172	[20] 38, 39	oxidase
evrL	31,941...32,882*	[25] 48, 49	heme biosynthesis
evrM	33,167...34,405*	[26] 50, 51	p450 hydroxylase
evrN	34,449...35,210*	[27] 52, 53	methyl transferase
evrQ	36,998...38,026*	[30] 58, 59	oxidoreductase/heat stress protein
evrT	40,216...40,890	[33] 64, 65	L-proline hydroxylase
evrU	40,887...41,576	[34] 66, 67	methyltransferase
evbA	53,554...54,207	[43] 84, 85	o-methyltransferase
evbE	58,873...60,312	[48] 94, 95	IMP dehydrogenase
evbI	66,469...67,872*	[54] 106, 107	lipoamide dehydrogenase
evbL	69,610...70,359*	[57] 112, 113	acetyltransferase/phosphotransferase
evbX	85,909...87,342	[69] 136, 137	aldehyde dehydrogenase
[exbY] evbY	87,422...88159	[70] 138, 139	aldehyde dehydrogenase
evcB	89,817...91,067	[73] 144, 145	cytochrome D oxidase subunit I
evcC	91,078...92,085	[74] 146, 147	cytochrome D oxidase subunit II

Please insert Table 6 on page 37 with the following Table: - -

Table 6. Regulatory Gene Products

Gene Product	CDS	SEQ ID No.	Enzymatic Function
evrR	38,072...38,566	[31] 60, 61	hexaheme nitrite reductase regulator/ methyltransferase
evsA	47,156...49,234*	[40] 78, 79	serine-threonine kinase
evbF	60,472...61,029*	[49] 96,97	
evbF2	61,610...62,069	[51] 100, 101	
evbK	68,529...69,494*	[56] 110, 111	protease synthase/sporulation regulator
evbR	76,622...78,712	[63] 124, 125	protein serine-threonine kinase (eukaryotic type)
evcJ	100,733...101,326*	[81] 160, 161	ATP/GTP binding protein
ORF1	189...1064*	[96] 183, 184	Transcriptional regulator biotinylation
ORF4	3776...4276*	[99] 189, 190	ECF sigma factor

- -

Please replace Table 7 on page 37 with the following Table:

- -**Table 7. Resistance Mechanism Genes**

Gene Product	CDS	SEQ ID No.	Enzymatic Function
evrE	19,374...20,906	[18] 34, 35	multidrug eflux transporter
evrY	45,014...45,760*	[38] 74, 75	dehalogenase
evrZ	45,962...46,714*	[39] 76, 77	muramidase/lysozyme
evbB	54,362...55,117*	[44] 86, 87	membrane pump

- -

Please replace the remainder of Table 7 on page 38 with the following Table: - -

evbC	55,135...56,094*	[45] 88, 89	membrane pump
evbC2	56,184...56,813*	[46] 90, 91	ankrylin-like
evbG	62,122...63,795	[52] 102, 103	ABC transporter
evbH	63,891...65,828	[53] 104, 105	ABC transporter
evcD	92,148...93,833	[75] 148, 149	ABC transporter
evcE	93,830...95,671	[76] 150, 151	ABC transporter
evrMR	107,653...108,615	[87] 172, 173	23S rRNA methylase
[evrMR12] evrMR2	108,635...109,216	[88] 174, 175	
ORF6	5392...6147*	[101] 193, 194	rRNA methyltransferase

- -

Please replace the 1st full paragraph of page 44 (lines 6-13) with the following paragraph:

- -In a specific embodiment, the present invention relates to a new nucleic acid sequence, to vectors for its expression and to its use in fermentation processes in actinomycetes. This nucleic acid sequence encodes a *Micromonospera*, and particularly *M. carbonacea, var. africana,* att/int functions and thus permits development of an integrating vector. In a specific embodiment, the att/int functions has an amino acid sequence as depicted in SEQ ID NO:[90] 177. In a more specific embodiment, the integrase is encoded by a nucleic acid having a nucleotide sequence as depicted in SEQ ID NO:[89] 176 (Figure 7B). A preferred integrating plasmid is shown in Figure 7A.- -

Please replace the last full paragraph on page 48 with the following:

- -The coding region of *evrF* gene was amplified with PCR primers:

5' PR 657 CCC TCG AGA TGT CCA GCA AGA TCC TA (SEQ ID NO: [91] 178);

3' PR 658 CGA ATT CTC AGG CAG ACT GCT CTG (SEQ ID NO: [92] 179); and

5' PR 659: CCC TCG AGA ATG TCC AGC AAG ATC CTA (SEQ ID NO: [93] 180);

3' PR 660: CGA ATT CAG ACT GCT CTG CCG CCG C (SEQ ID NO: [94] 181);

using the Advantage-GC Genomic PCR kit and Advantage HF polymerase (Clontech, Palo Alto, CA) and a Perkin-Elmer 9600 PCR machine (Foster City, CA). The 1.5 kb PCR products were digested with *XhoI* and *EcoRI* and the fragments were ligated to *XhoI* and *EcoRI* digested pBADHisA (primer pair PR657/PR658 product) and pBADMycHisC (primer pair PR659/PR660 product) and transformed into *E. coli* Top10 (Stratagene, LaJolla, CA). Transformants were analyzed by plasmid isolation followed by digestion and gel electrophoresis analysis. Appropriate clones were also verified by sequence analysis. This yielded the evrF expression clones pSPRE59 (pBADHisA) and pSPRE19 (pBADMycHisC). Top10 cells containing either pSPRE59 and pSPRE19 were grown overnight at

37°C with shaking in LB containing 50ug/ml AMP. Overnight cultures were used to innoculate fresh LB containing 50 μg/ml and grown at 37 °C with shaking to an OD_{600} of 0.4 to 0.5. L-arabinose was added to a final concentration of 0.02% and the culture was incubated for an additional 4 hours. Cells were collected by centrifugation, resuspended in 100 μl Tris-Glycine buffer and boiled for five minutes. Whole cell protein lysate was loaded onto a SDS-PAGE gel, electrophoresed, and stained with coomassie blue to determine protein expression.- -